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METHODS FOR DIAGNOSING GLAUCOMA AND DISCOVERING ANTI-GLAUCOMA DRUGS

by

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This application is a continuation-in-part of U.S. application Serial No. 09/308,295, filed May 17, 1999, which is a 371 application of PCT/US97/21054, filed November 14, 1997. Priority is claimed from the provisional application, U.S. Patent Application Serial No. 60/033227 filed December 5, 1996.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to the field of glaucoma diagnosis and treatment. More specifically, the present invention provides methods for diagnosis of glaucoma by measuring the amount of GRβ present in the trabecular meshwork of a patient's eye.

2. Description of the Related Art

Glaucoma is usually diagnosed by monitoring a patient's visual field loss, changes in the appearance of their optic disc, and their intraocular pressure. Glaucoma is currently treated using one or more of three strategies to lower the elevated intraocular pressure associated with the disease: with pharmaceuticals (such as beta-blockers, carbonic anhydrase inhibitors, miotics or prostaglandins), with laser trabeculoplasty, and/or with glaucoma filtration surgery. All of these therapies indirectly lower intraocular pressure but do not address the underlying disease process occurring in the trabecular meshwork. It would be advantageous to be able to diagnose glaucoma before a patient begins experiencing a loss in their visual field and deterioration of their optic disc.

There is a large body of evidence suggesting that glucocorticoids are involved in the generation of ocular hypertension and glaucoma (Clark 1995). The human glucocorticoid receptor (hGR) and its isoforms, hGRα (SEQ ID NO:3) and hGRβ (SEQ

ID NO:1), are described in Encio and Detera-Wadleigh (1991) (See also FIG. 1). Several investigators have shown that the human trabecular meshwork (TM) contains the classical glucocorticoid receptor (hGRα) (Weinreb *et al.* 1981; Hernandez *et al.* 1983). Recently, the expression of an alternatively spliced form of the human glucocorticoid receptor (hGRβ) was discovered in non-ocular tissues and cells (Bamberger *et al.* 1995; Oakley *et al.* 1996). This alternatively spliced form of hGR is expressed as a protein (SEQ ID NO:2) which no longer binds glucocorticoids, but is able to interfere with the activated form of the normal glucocorticoid receptor and block or alter physiological functions of the glucocorticoid receptor.

SUMMARY OF THE INVENTION

The present invention is directed to methods for diagnosing glaucoma by testing a person for aberrant hGRβ expression. In preferred embodiments, a decrease in hGRβ expression in the trabecular meshwork of glaucomatous eyes as compared to hGRβ expression in non-glaucomatous eyes. Also set forth are methods for screening for therapeutic agents useful for treating glaucoma.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawing forms part of the present specification and is included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to this drawing in combination with the detailed description of specific embodiments presented herein.

FIG. 1. Illustrates the alternative splice forms of the human glucocorticoid receptor (hGR) gene.

FIG. 2. Illustrates binding of glucocorticoids to GRα and failure of glucocorticoids to bind to GRβ.

DESCRIPTION OF PREFERRED EMBODIMENTS

Surprisingly, it has been found that cultured human trabecular meshwork cell lines express the proteins from both an alternate splice form of the human glucocorticoid receptor (GRβ; SEQ ID NO:1), as well as the normal glucocorticoid receptor (GRα; SEQ ID NO:3). Glaucomatous TM cells have less GRβ protein and therefore are more susceptible to endogenous and exogenous glucocorticoids. It is believed that the elevated intraocular pressure associated with primary open-angle glaucoma may be due to the aberrant expression of GRβ in the trabecular meshwork. Therefore, determining that an individual abnormally expresses GRβ in their trabecular meshwork or other tissues can lead to a diagnosis of glaucoma.

The present invention further provides a method for determining whether a candidate substance has therapeutic value in treating glaucoma by determining whether the candidate substance interacts with the GRβ protein (SEQ ID NO:2) or alters the expression of GRβ (SEQ ID NO:1). This can be done using ligand binding assays or GRβ functional assays.

Diagnosing aberrant GRβ expression or defects in the GR gene which encodes GRβ can be done by using procedures well known to those skilled in the art (Caskey 1993). For example, subjects could be screened for the presence of a genetic defect in GRβ by analyzing the DNA derived from peripheral blood leukocytes. Types of DNA analyses could include, but would not be limited to: restriction fragment length

polymorphisms (RFLP), single-stranded conformation polymorphisms (SSCP), polymerase chain reaction (PCR), denaturing gradient gel electrophoresis, allele specific oligonucleotide ligation assay, and allele specific hybridization assay. In addition, trabecular meshwork, or other relevant cells from subjects could be analyzed for GRβ expression by a number of techniques such as reverse-transcription polymerase chain reaction (RT-PCR), immunoassays, GR functional assays, etc.

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1

Effect of GRβ transfection on DEX-induced myocilin expression in TM cells

GRα and GRβ expression vectors and specific antibodies were generated. The expression and localization of GRα and GRβ in normal and glaucomatous TM cell lines was examined in cells containing dexamethasone (DEX) and in cells lacking DEX.

It was found that GRβ is expressed at higher levels in normal (non-glaucomatous) TM cells. It exists in both the cytoplasm and in nucleus (IF) and is more concentrated in the nucleus. In glaucomatous TM cells, the amount of GRβ is relatively lower than in

normal TM cells. It was also noted that GRβ is evenly distributed in the cytoplasm and nucleus of glaucomatous TM cells.

Treatment with DEX caused GRα nuclear translocation into the nucleus. DEX time-dependently down-regulated GRα in both normal and glaucomatous TM cells. Western blotting detected GRβ doublets present in both cytoplasm and nuclear region. Three day DEX treatment increased nuclear short form of GRβ in normal TM cell lines, but not in glaucomatous TM cell lines. Both GRα and GRβ co-precipitate with Hsp90. It was also found that GRβ blocks DEX induction of MYOC.

All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and structurally related may be substituted for the agents described herein to achieve similar results. All such substitutions and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

References

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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